

AMENDMENT TO SPECIFICATION

Please amend the paragraph in Table 1 on page 6, line 11 as follows:

Initial searches were done using the HERV database ~~http://HERV.img.cas.cz~~ HERV.img.cas.cz. The four query sequences correspond to all four insertion orientations into the two host DNA strands. Query A and B yielded no hit. Identities value correspond to the region of best match. Chromosomal location was confirmed by repeat searches using Genbank. No homologies between the queries and known expressible human proteins was obtained in these searches.

Please amend the paragraph beginning on page 24, line 20 as follows:

V_LK back (Sfi I site underlined)-

SEQ ID NO 7:

GTCCTCGCAACTGCGGCCCCAGCCGGCCATGGCCGACATCCAGATGACCCAGTCTCC,

SEQ ID NO 8:

GTCCTCGCAACTGCGGCCCCAGCCGGCCATGGCCGATGTTGTGATGACTCAGTCTCC,

SEQ ID NO 9:

GTCCTCGCAACTGCGGCCCCAGCCGGCCATGGCCGAAATTGTGTTGACGCAGTCTCC,

SEQ ID NO 10:

GTCCTCGCAACTGCGGCCCCAGCCGGCCATGGCCGACATCGTGATGACCCAGTCTCC,

SEQ ID NO 11:

GTCCTCGCAACTGCGGCCCCAGCCGGCCATGGCCGAAACGACACTCACGCAGTCTCC,

SEQ ID NO 12:

GTCCTCGCAACTGCGGCCCCAGCCGGCCATGGCCGAAATTGTGCTGACTCAGTCTCC; CK

forward (Not I site underlined): SEQ ID NO: 13:

CCATCCTGCGGCCGCACACTCTCCCCTGTTGAAGCT

Please amend the paragraph on page 25 line 1 as follows:

(b) Human single chain Fv: V.sub.LK back- see back primers, full-length L chain; V_LK forward (Xho I site underlined) –

SEQ ID NO: 14: GCCTGAACCGCCTCCACCACTCGAGCGTTTGATTTCACCTTGGTCCC,

SEQ ID NO: 15:

GCCTGAACCGCCTCCACCACTCGAGCGTTTGATCTCCAGCTTGGTCCC,

SEQ ID NO: 16:

GCCTGAACCGCCTCCACCACTCGAGCGTTTGATATCCACTTTGGTCCC,

SEQ ID NO: 17:

GCCTGAACCGCCTCCACCACTCGAGCGTTTGATCTCCACCTTGGTCCC,

SEQ ID NO: 18:

GCCTGAACCGCCTCCACCACTCGAGCGTTTAATCTCCAGTCGTGTCCC;

V_Llamda. back (Sfi I site underlined) –

SEQ ID NO: 19:

GTCCTCGCAACTGCGGCCCCAGCCGGCCATGGCCCAGTCTGTGTTGACGCAGCCGCC,

SEQ ID NO: 20:

GTCCTCGCAACTGCGGCCCCAGCCGGCCATGGCCCAGTCTGCCCTGACTCAGCCTGC,

SEQ ID NO: 21:

GTCCTCGCAACTGCGGCCCCAGCCGGCCATGGCCTCCTATGTGCTGACTCAGCCACC,

SEQ ID NO: 22:

GTCCTCGCAACTGCGGCCCCAGCCGGCCATGGCCTCTTCTGAGCTGACTCAGGACCC,

SEQ ID NO: 23:

GTCCTCGCAACTGCGGCCCCAGCCGGCCATGGCCCACGTTATACTGACTCAACCGCC,

SEQ ID NO: 24:

GTCCTCGCAACTGCGGCCCCAGCCGGCCATGGCCCAGGCTGTGCTCACTCAGCCGTC,

SEQ ID NO: 25:

GTCCTCGCAACTGCGGCCCCAGCCGGCCATGGCCAATTTTATGCTGACTCAGCCCCA;

V_Llamda. forward (Xho I underlined) –

SEQ ID NO: 26:

GCCTGAACCGCCTCCACCACTCGAGCCTAGGACGGTGACCTTCGTCCC,

SEQ ID NO: 27:

GCCTGAACCGCCTCCACCACTCGAGCCTAGGACGGTCAGCTTGGT CCC,

SEQ ID NO: 28:

GCCTGAACCGCCTCCACCACTCGAGCCTAAAACGGTGAGCTGGGTCCC;

C_Llamda. forward SEQ ID NO: 29: - TGAAGATTCTGTAGGGGCCACTGTCTT;

V_H back (ApaL site underlined) –

SEQ ID NO: 30: CATGACCACAGTGCACTTCAGGTGCAGCTGGTGCAGTCTGG,

SEQ ID NO: 31: CATGACCACAGTGCACTTCAGGTCAACTTAAGGGAGTCTGG,

SEQ ID NO: 32: CATGACCACAGTGCACTTGAGGTGCAGCTGGTGGAGTCTGG,

SEQ ID NO: 33: CATGACCACAGTGCACTTCAGGTGCAGCTGCAGGAGTCGGG,

SEQ ID NO: 34: CATGACCACAGTGCACTTCAGGTGCAGCTGTTGCAGTCTGC,

SEQ ID NO: 35: CATGACCACAGTGCACTTCAGGTACAGCTGCAGCAGTCAGG;

V_H forward (Not I site underlined) –

SEQ ID NO: 36: GAGTCATTCTGCGGCCGCGGGGAAGACSGATGGGCCCTTGGT,

SEQ ID NO: 37:GAGTCATTCTGCGGCCGCGGGGAAAAGGGTTGGGGCGGATGC;

Please amend the paragraph on page 11, line 21 as follows:

HERV sequences with homology to modern-day microbial proteins are identified by database searches, available, for example at Blastn and the HERV database, ~~http://herv.img.cas.cz~~ herv.img.cas.cz. DNA sequences corresponding to antigenic epitopes 5-7 of the microbial antigenic target are first identified, as the minimum length of an antigenic epitope is generally thought to be 5-7 amino acids (15-21 nucleotides). The epitope to be targeted is generally selected based on its functional importance. For example, in the case of HIV, it is beneficial to choose an antigenic epitope blockade of which can be expected to result in inhibition of binding to host cell CD4 receptors, and, consequently, inhibition of viral entry into host cells. The level of statistical significance for determination of homology with the query sequence depends on several factors, including the number of nucleotides that are identical to the query and the number of gaps that in the identities. Several software programs are available to judge the significance of the homology (e.g., ref 23). Equally important when assessing homology is the likelihood of structural similarity between the query peptide epitope and the HERV peptide epitope. For example, dissimilarities at certain amino acids can result in large structural changes, e.g., introduction of a Pro residue can disrupt the helical structure of an epitope. Reference 24 describes an algorithm to assess peptide sequences based on the chemical similarity of their component amino acids. This algorithm can be employed to identify the best HERV candidate antigens for the isolation of Abs.

Please amend the paragraph in Table 4 on page 28 as follows:

R - Replacement mutations: S - Silent mutations. *, number of amino acids. Germline counterparts identified from ~~http://www.ncbi.nlm.nih.gov/igblast~~ www.ncbi.nlm.nih.gov/igblast. CDRs identified by comparison with Kabat database. Mutation counts restricted to 3' termini of V genes. FR1 residues 1-7 excluded because these are encoded by PCR back primers. Family and subgroup assignment from ~~http://immuno.bme.nwu.edu/~~ immuno.bme.nwu.edu/. cDNA sequences determined in the 5' and 3' directions were identical. Alignments with # germline V and J genes suggested extensive diversification due to V-(D)-J recombination. For this reason, germline D genes were unassignable. Fv JL413 contained 20 and 17 deletions at the VH gene 3' end and J gene 5' end, respectively.

Please amend the paragraph beginning on page 42, line 9 as follows:

Total RNA and MnRNA from healthy individuals and uninfected lupus patients positive for Abs (N=20 each) that bind gp120 at residues 422-432 are prepared by standard phenol extraction and oligo-dT purification methods taking care to minimize ribonuclease digestion of the RNA. Reverse transcriptase-polymerase chain reaction (RT-PCR) is used to amplify the desired cDNA. The forward primer is oligo-dT (15 mer). As examples, the following two back primers are tested: (a) the primer corresponding to the consensus sequence of gp120 residues 422-432 [caaattataaacatgtggcaggaagtaggaaaa]; and (b) the primer corresponding to the sequence of HERV-L region homologous to gp120 residues 422-432 [caaattaaaaactttttaagaaagtaggaaaa]. RT-PCRs conducted with PBMCs from HIV+ subjects serves as the positive control. This yields a well defined PCR product because of expression of the gp120 gene in infected cells. Genomic DNA is analyzed in parallel to determine whether nonexpressible sequences homologous to residues 422-432 are present. A primers complementary to a non-expressed gene sequence (e.g., an intron sequence) is used as the negative control to assure specificity of amplification. The reactions are carried out at several temperatures and MgCl₂ concentrations to vary annealing permissivity and allow annealing despite partial mismatches with the template. The size of the cDNA product depends on the number of nucleotides in the mRNA separating the poly A tail from the gp120 422-432 region. We have previously applied similar degenerate annealing methods to amplify Ab V sequences, which contain homologous but non-identical 5' ends. Once a well-defined PCR product has been demonstrated, it is sequenced. For this purpose the PCR product is cloned into a suitable vector [e.g., cloning via Sfi I and Not I restriction sites into pCANTAB5His6 followed by conventional didexynucleotide sequencing]. Homology analyses identifies the gene from which the PCR product is derived [Blastn; HERV database, <http://herv.img.cas.cz> ~~http://herv.img.cas.cz~~ herv.img.cas.cz; as noted above, the PCR product corresponds to a gene fragment].

Please amend the paragraph beginning on page 43, line 24 as follows:

Further immunological and genetic analyses: Further experimental maneuvers are dictated by the results of the preceding studies. For example, the cDNA identified in the preceding section could correspond to a previously characterized, full-length HERV protein. Another scenario is that a relatively short HERV element homologous to residues 422-432 is inserted into a gene encoding a non-HERV protein. For illustration, following is a brief description of certain general methods. The gene fragment identified to be homologous to gp120 is radiolabeled with [³²P]dCTP using a commercially available kit and applied as a

hybridization probe to screen a cDNA library such as the human leucocyte expression library in λ phagemid available from Clontech [obtained from human RNA pooled from 585 Caucasians; contains long cDNA inserts >3 kbases, mostly corresponding to full-length genes]. If the desired gene is found to be expressed only in lupus patients, a new cDNA library from PBMC of lupus patients is constructed as in (8). Standard hybridization methods can be applied to identify and sequence the clone(s) annealing with the probe, which helps identify the full-length gene encoding the peptide determinant homologous to gp120 residues 422-432. Following confirmation of the presence of an open reading frame (~~<http://www.ncbi.nlm.nih.gov/gorf/gorf.html>~~ www.ncbi.nlm.nih.gov/gorf/gorf.html), the cDNA corresponding to the full-length is recloned in an appropriate expression vector [e.g., baculovirus system to ensure appropriate post-translational processing; a his6 tag can be introduced to allow rapid purification].